Glycoconjugates Characterization in Jejunal Goblet Cell of the Chicken by Means of Lectin Histochemistry

Prapassorn Boonsoongnern, Krissana Saengprapaitip, Dollada Srisai, and Apinun Suprasert

ABSTRACT

The labeling pattern of seven lectins was studied in order to define the normal distribution of secretory glycoconjugates in jejunal goblet cells of the chicken. The following 7 lectins were employed by means of a peroxidase technique on formalin, Carnoy’s or Rossman’s fixed sample: Concanavalin A (Con A), *Ricinus communis* agglutinin–I (RCA-I), *Dolichos biflorus* agglutinin (DBA), Wheat germ agglutinin (WGA), *Ulex europaeus* agglutinin-I (UEA-I), Peanut agglutinin (PNA), and *Limax flavus* agglutinin (LFA).

The positive staining with Con A, RCA-I and WGA in goblet cells was found to increase during upward migration from crypt to villus. PNA reacted with mucous granules of goblet cells throughout the villus and crypt axis. DBA, UEA-I and LFA lectin labeling of goblet cells appeared to be negative.

This finding suggests that during cell migration from crypt to villus tip, the maturation of goblet cells was associated with the differentiation of secretory glycoconjugates.

Key words: jejunal goblet cells, glycoconjugates, lectin histochemistry
INTRODUCTION

Goblet cells are the mucus-secreting epithelial cells and are particularly abundant in the intestinal epithelium. Glycoconjugates in the intestinal epithelium have been studied in several mammals. Spicer (1960) reported on the histochemical nature of glycoconjugates of intestinal goblet cells in guinea pig, rabbit, mouse and rat. Subbusway (1971) described the normal distribution of glycoconjugates in the human intestinal goblet cells. Sheahan and Jervis (1976) compared the histochemistry of gastrointestinal mucosubstances of 11 mammals. Poddar and Jacob (1979) studied the nature of mucosubstances in the duodenal goblet cells of the ferret. Freeman et al (1980) applied the lectins to detect particular sugars in colonic goblet cell of the rat. Recently, furthermore, the glycoconjugates histochemistry also has been studied in the gastrointestinal epithelium of fish and frog (Ferri et al., 2001, Pendini et al., 2005). These studies suggested considerable interspecies variation in a given organ and regional variation even within a species in the histochemical reactivity of glycoconjugates of goblet cell. In the chicken small intestinal goblet cell, however, there have been no reports to date on histochemistry of glycoconjugates.

In view of the circumstances mentioned the distribution of structural and secretory glycoconjugates in chicken jejunal goblet cells remain to be defined. A more complete knowledge of their distribution may help to set the stage of further studies. In this investigation, a large number of lectins were employed in order to gather as much information as possible about the characteristics of lectin labeling under carefully controlled experimental conditions.

MATERIAL AND METHODS

10 male adult Brown Leghorn chickens were used. Tissue samples were taken from the middle part of the jejunum. They were fixed with one of the following fixatives: (1) 10% formalin in 2% calcium acetate for 12-24 h at 4°C. (2) Rossman’s fluid for 12 h at 4°C. (3) Carnoy’s fluid for 4-6 h at room temperature. Thereafter, they were dehydrated in a grade ethanol series of ascending concentration and embedded in paraffin. Serial sections were cut at a thickness of 3 μm and then subjected to stain with the conventional and lectin staining procedures.

Conventional staining procedures

1. Haematoxylin and eosin (H&E) for the general observation of histological structure.
2. Periodic acid-Schiff (PAS) for vicinal diol containing glycoconjugates (Pearse, 1968)
3. Alcian blue (AB) pH 2.5 for acidic glycoconjugates (Spicer et al., 1967).
4. AB pH 2.5-PAS for demonstrating of acidic and neutral glycoconjugates (Spicer et al., 1967)
5. High iron diamine (HID) for sulfated glycoconjugates (Spicer 1965).
6. HID-AB pH 2.5 for differentiating sulfated and carboxylated glycoconjugates

Lectin staining procedures
1. Concanavalin A (Con A) for glucose or mannose residues of glycoconjugates.
2. *Ricinus communis* agglutinin (RCA-I) for galactose residues of glycoconjugates (Goldstein and Hayes, 1978).
3. Wheat germ agglutinin (WGA) for detection of N-acetylglucosamine of glycoconjugates (Goldstein and Hayes, 1978)
4. *Dolichos biflorus* agglutinin (DBA) for N-acetylgalactosamine residues of glycoconjugates (Goldstein and Hayes, 1978)
5. *Ulex europeus* agglutinin (UEA-I) for detection of fucose residues of glycoconjugates (Goldstein and Hayes, 1978)
6. Penut agglutinin (PNA) for detection galactose-(1-3) N-acetylgalactosamine disaccharides of glycoconjugates (Stoward, 1980).
7. *Limax flavus* agglutinin (LFA) for detection of sialic acid residues of glycoconjugates (Schulte et al, 1984)

Control staining for lectin
Lectin containing a particular sugar was performed as control staining procedures. In order to detect the activity of endogenous peroxidase in tissue, some control tissue sections were reacted with DAB only.

RESULTS

In the chicken jejunum, the staining reactivities in jejunal goblet cells were optimal in the specimens fixed with Carnoy’s and Rossman’s fluid, but unsatisfactory in those fixed with formalin-calcium.

The goblet cells and striated border of columnar cells in the chicken jejunum were PAS-positive and were stained bright red. The both structures stained weakly with AB pH 2.5. When stained with HID, the goblet cells and striated border of columnar cells turned black. The combined staining with both AB pH 2.5-PAS and HID-AB pH 2.5 resulted in strong positive reaction (Fig. 1 and 2).

When the jejunal epithelium was reacted with lectin, the goblet cells and striated border of columnar cells reacted strongly with RCA-I (Fig. 3) and PNA (Fig 6), and moderately with Con A and WGA(Fig 4). The positive staining with Con A, RCA-I and WGA was found to increase in intensity from the crypt to villus tip. The goblet cells were, however, negative to DBA, UEA-I (Fig 5) and LFA.

All the results obtained from conventional and lectin staining procedures were summarized in Table

DISCUSSION

The arrangement of jejunal goblet cells was similar to that of most mammals (Spicer, 1960). The histochemical reactions showed that the goblet
Table 1  Histochemical reaction of glycoconjugates in goblet cells of the chicken jejunum.

<table>
<thead>
<tr>
<th>Staining procedures</th>
<th>Crypt goblet cell</th>
<th>Villus goblet cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td>2 M</td>
<td>2 M</td>
</tr>
<tr>
<td>AB pH 2.5</td>
<td>1B</td>
<td>1B</td>
</tr>
<tr>
<td>HID</td>
<td>1-2 Bl</td>
<td>1-2 Bl</td>
</tr>
<tr>
<td>AB pH 2.5-PAS</td>
<td>2-3 MB</td>
<td>2-3 MB</td>
</tr>
<tr>
<td>HID-AB pH 2.5</td>
<td>2 BBl</td>
<td>3 BBl</td>
</tr>
<tr>
<td>Con A</td>
<td>1-2 Br</td>
<td>2-3 Br</td>
</tr>
<tr>
<td>RCA-I</td>
<td>1-2 Br</td>
<td>3-4 Br</td>
</tr>
<tr>
<td>WGA</td>
<td>0-1 Br</td>
<td>2-3 Br</td>
</tr>
<tr>
<td>DBA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UEA-I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PNA</td>
<td>3 Br</td>
<td>3-4 Br</td>
</tr>
<tr>
<td>LFA</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
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**Abbreviation**  B = Blue, Bl = Black, Br = Brown, M = Magenta, 0 = Negative reaction, 1-n = Number indicates intensity of staining reaction.

**Fig. 1**  In the chicken jejunal epithelium, villus goblet cells and crypt goblet cells were stained moderately with AB pH 2.5-PAS.

**Fig. 2**  Mucous granule in crypt and villus goblets cells, and striated border of columnar cells exhibited strong reaction. HID-AB pH 2.5 staining
Fig. 3  As in Fig. 2 RCA-I staining

Fig. 4  Villus and crypt goblet cells were weakly reactive. WGA staining.

Fig. 5  Villus and crypt goblet cells exhibited negative reaction. UEA-I staining.

Fig. 6  Villus and crypt goblet cells were stained moderately with PNA.
cells stained acidic glycoconjugates, since mucous grannules of the cells were stained pale blue with AB pH 2.5. In addition, the goblet cells contained sulfate groupings, as indicated from the positive reaction with HID (Spicer, 1965). In the combined AB pH 2.5-PAS method, most goblet cells were deep blue, but some others appeared reddish purple. The purple color is considered to be due to the mixture of neutral and acidic glycoconjugates (Spicer et al., 1967). Alcian blue is generally regarded as being specific for identify the carboxyl groups of sialoglycoconjugates or other glycoconjugates with a strong negative charges such as sulfate groups while PAS is known to stain sugar with vicinal diol groups and a neutral charge, including galactose, mannose and fucose residues (Spicer and Schulte 1992).

Lectin staining procedure has revealed some differences in the localization of the glycoconjugates. In the present study, the jejunal goblet cell and striated border of columnar cells stained intensely with PNA, thereby suggesting that mucous granules of goblet cells and microvillar surface of columnar cells are rich in glycoconjugates with terminal galactose-(1-3) N-acetylgalactosamine disaccharides (Stoward et al., 1980). Lectin staining also showed the variation in quantity of carbohydrate in goblet cells between the crypt and the villi by the color of the staining intensity of -D-mannose, -D-galactose and N-acetylglucosamine with Con A, RCA-I and WGA respectively. These finding suggest that, during cell migration from crypt to villus tip, the continuous maturation of goblet cells was associated with the differentiation of secretory glycoconjugates. However, DBA, UEA-I and LFA lectin labeling of goblet cells appeared to be negative.

The histochemical patterns of distribution in the epithelial glycoconjugates of gastrointestinal tract of the various species studied showed remarkable differences despite the similarity of their morphologic appearances. The presences of sulfated glycoconjugates in the chicken jejunal goblet cells were in contrast to that of human small intestinal goblet cells (Filipe and Fenger, 1979). Furthermore, our finding about negative LFA staining of goblet cells differs from most mammalian small intestinal goblet cells which contained terminal sialic acid residues (Schulte et al., 1984). The absent of fucose residues in the chicken jejunum was also opposite to that of in the frog and fish intestinal goblet cells. (Pendini et al., 2002, 2005).

In conclusion, our data define the normal picture of lectin labeling in jejunal goblet cells. More data is needed in order to better define the significance of these findings. However, lectin histochemistry appears reliable in the study of secretory and structural glycoconjugates of cellular differentiation in course of jejunal cell maturation.

REFERENCES